Observations on Trypanoplasma congeri.

Part I.—The Division of the Active Form.

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With Plate 21, and 1 text-figure.

CONTENTS.

GENERAL INTRODUCTION.

In the 'Zoologischer Anzeiger,' Bd. xxxv, Nos. 14 and 15, Mr. Elmhirst and I published a short note on a trypanoplasma parasitic in the stomach of the conger eel (Conger niger). Up to the present, as far as I am aware, no satisfactory account has been given of the division of any trypanoplasma, and the only point in connection with this process, on which previous workers have been unanimous, seems to be the extreme rarity of dividing forms. By what I must now regard as rather a fortunate accident, the second conger which I chanced to examine was so heavily infected that frequently two dividing forms have been found in the same field. As such infections, however, seem extremely rare, I have decided to publish my observations on the division of the active form of *Trypanoplasma congeri* at once, reserving the notes we have at present made on the changes into the resting form for a later paper. In the later paper we hope also to deal more fully with the general literature of the group. I shall only mention in this paper the previous accounts of intestinal trypanoplasma, and, in a later section of the paper, I shall refer to the descriptions of division given for this genus by Keysselitz and Friedrich. The first description of an intestinal trypanoplasma was given by Lèger in 1905 for a form, *Trypanoplasma intestinalis*, which he found in the stomach of *Box boops*.

The second intestinal trypanoplasma, hitherto described *Trypanoplasma ventriculi*, was found by Keysselitz in the stomach of *Cyclopterus lumpus*, and is figured on p. 37 of his paper on Generations- und Wirtswechsel von *Trypanoplasma borreli*.

I do not propose to enter into any details as to the conditions under which *Trypanoplasma congeri* occurs in this paper, as these notes will be reserved for our later paper. The active form of the parasite is, however, always found in sections of the conger's stomach in the mucus lining the surface of the wall, and it never seems to spread into the deep glandular pits. Up to the present no sign of the active trypanoplasma has been found in any part of the intestine or rectum, and, in fact, if active trypanoplasma are mounted in the intestinal juice they almost immediately become agglomerated by their posterior extremities, and have disappeared entirely at the end of a couple of hours. Up to the present forty-seven congers have been examined, and of these only ten have been found to be infected. The parasite has been found in small numbers in some congers in which the stomach and intestine were full of food, but the only really heavy infections have been obtained from fasting congers.

I should like to take this opportunity of thanking Mr. Elmhirst, the director of the Marine Station at Millport, for his assistance in getting material, and Miss Robertson for help in the drawing of the figures.

Methods.

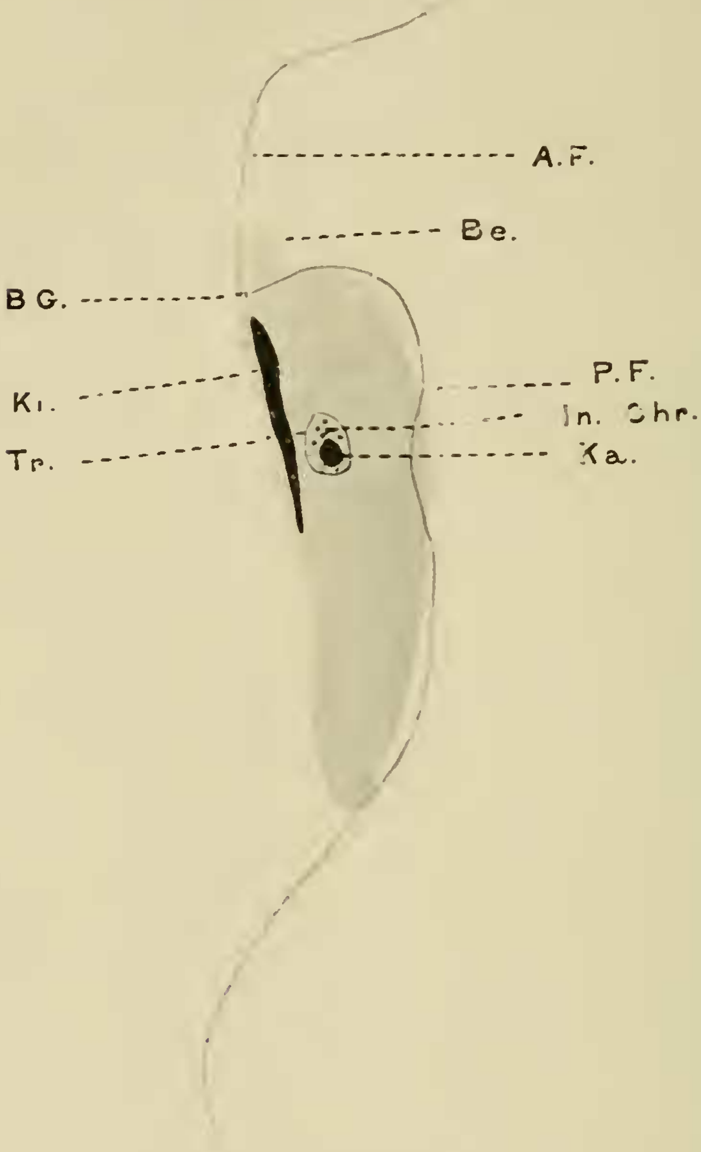
The stages figured in this paper were all obtained on wet smears from the stomach wall, fixed either in Flemming or corrosive acetic. Both of these methods gave excellent results. The films were stained in Giemsa, Twort, iron-hæmatoxylin and eosin, and Mayer's acid hæmalum and eosin. All these stains gave satisfactory results, but the figures were all drawn from preparations made either with hæmalum and eosin, or iron-hæmatoxylin and eosin.

Morphology of the Active Form.

As there seems to be a certain amount of discrepancy amongst different authors in regard to the nomenclature of the various structures in trypanosomes and trypanoplasmas, I have indicated in the following diagram the nomenclature I have decided to adopt. It is practically that used by Minchin in his paper on the structure of Trypanosoma lewisi in relation to microscopical technique ('Quart. Journ. Micros. Sci.,' vol. 53, 1909, p. 799).

The normal active Trypanoplasma congeri has rather an elongate body, measuring roughly $18\,\mu$ by $2{\cdot}7\,\mu$. The two flagella arise apparently from a single basal granule near the anterior end of the kinetonucleus; the anterior flagellum passes up the mobile beak to end freely, while the posterior flagellum passes transversely across the body of the animal, and running down in connection with the narrow undulating membrane, projects freely for a distance of about $10\,\mu$ beyond the animal's posterior end. As regards the basal granule, most previous observers seem to have been of the opinion that each flagellum in Trypanoplasma arises from a separate basal granule, although it is evident that they do not regard the matter as absolutely certain, e.g. Minchin, in his

TEXT-FIG. 1.—Active form of Trypanoplasma congeri.



A. f. Anterior flagellum. *Be.* Beak. *B. g.* Basal granule. *In. chr.* Intra-nuclear chromatin. *Ka.* Karyosome. *Ki.* Kinetonucleus. *P. f.* Posterior flagellum. *Tr.* Trophonucleus.

paper on the blood-parasites of fish, remarks as regards Trypanoplasma keysselitzi, p. 28, "In front of the kinetonucleus are situated the two minute blepharoplasts, from which the flagella arise. I believe them to be always two in number, but in iron-hæmatoxylin preparations they are so minute and often so close together that it is impossible to resolve them as two granules, and they may appear as a single dot."

In the active Trypanoplasma congeri the two flagella always appear to me to arise from a single basal granule, and from what I have seen of the dividing and resting forms I am certain that if the flagella do not arise from a single basal granule, the connection between the two granules must be so intimate that the flagella always behave as though they arose from a single point. Passing down the side of the animal under the membrane a row of very faintly staining rounded granules are frequently seen; these may correspond to the structures described in Trypanophis, or possibly to the far more strongly staining granules seen in some forms of Trichomonas. The trophonucleus in the elongate form of Trypanoplasma congeri lies about one third of the animal's length from the anterior end, and usually consists of a conspicuous membrane containing a darkly staining elliptical karyosome, which is usually surmounted at its anterior end by a cap of chromatin granules. In some cases, however, the karyosome is central and the granules are arranged round it. These appearances recall Schaudinn's figure of the chromosomes in the resting nucleus of Trypanomorpha and Leger's description of the chromosomes of Trypanoplasma intestinalis. It will, however, I think, become abundantly clear from the behaviour of the dividing trophonucleus described below that it is impossible to regard the chromatin granules of Trypanoplasma congeri as chromosomes.

The kinetonucleus is usually a very darkly staining carrot-shaped structure lying laterally near the animal's anterior end, the narrow posterior end of the kinetonucleus passing down the animal's body to end in the region of the trophonucleus. In some cases the kinetonucleus presents an almost segmented appearance, and apparently this appearance has in many cases been taken as an early indication of division, though I believe this interpretation to be erroneous.

Division.

All of the preparations here figured are taken from films of the stomach of a fasting conger which had been kept in the tanks at Millport for four months, and was killed at 5.30 p.m., November 27th. In the early stage of division (Pl. 21, fig. 2) the body of the animal becomes slightly shorter and thicker. The basal granule of the flagella divides, and this is followed by a splitting, first of the anterior flagellum along its whole length, and then of the posterior flagellum with its membrane. The trophonucleus and its contained karyosome become larger, and I believe that the intra-nuclear chromatin granules (? the "chromosomes" of Schaudinn) at this stage become condensed on to the karyosome. The kinetonucleus at this stage becomes slightly thicker, but shows no distinct indication of division. In the next stage (Pl. 21, fig. 3) the flagella have split along their whole length, and it is important to note that, in marked distinction to the state of affairs found by Friedrich in Trypanoplasma helicis, I have never been able to find the slightest evidence of the growth of new flagella in any stage of division. The trophonucleus now assumes a spindle shape, and the karyosome divides; the two halves, however, remaining connected by a rod, which persists until a very late stage of division. It might have been expected that some sign of the so-called chromosomes would be found at this stage lying around the dumb-bell-shaped karyosome in the spindle-shaped nucleus, but no trace of them has been detected. It is, of course, possible that this may be due to faulty technique, but so many of these dividing stages have been found lying near resting forms with nuclei clearly showing these granules that I believe this hypothesis is untenable. The relation of the axis of the trophonucleus spindle to the longitudinal axis of the animal's body seems in these early stages to be rather variable, but in the later stages the long axis of the spindle seems always to be arranged in direction transverse to the animal's original longitudinal axis. The kinetonucleus now becomes very much enlarged, and gradually (Pl. 21, figs. 4–7) pushes out a posterior limb, which comes to lie at right angles across the dumb-bell-shaped trophonucleus. This relation seems very characteristic of this stage of division, which is a very common one on these films. It is rather interesting to note that the stages of division up to this point in the films from this particular conger are very common, the latter stages being comparatively rare. As these films were taken from various points all over the surface of the stomach, this would seem to point either to a cyclical epidemic of division in this parasite or (a view which seems to me rather more improbable) to an extremely short duration for the later as compared with the earlier stages of division. The basal granules have now moved some distance apart, and as the animal shortens and thickens the membranes and flagella become shifted round till in the later stages they pass down the opposite sides of the body. The trophonucleus now is completely dumb-bell shaped, the handle of the dumb-bell being formed by the strand connecting the two karyosomes. In its early stages the dividing trophonucleus has presented a very superficial resemblance, in outline, at any rate, to the mitotic spindles found in the metazoan cell, but in the succeeding stages, in which the new trophonuclei have become definitely rounded, and their connection is limited to the bar joining the two karyosomes, this resemblance is completely lost. In Pl. 21, fig. 8, a late stage of division is figured in which the two products of division are still connected with each other by a narrowing band of protoplasm, through which, even at this stage, the kinetonuclei and trophonuclei are still connected. In Pl. 21, fig. 9, a form is shown which has evidently just divided. It is characterised firstly by its small size and rounded shape, secondly by the length of the kinetonucleus, and thirdly by the remains of the strand of the karyosome which had connected the two trophonuclei, and which has not yet been withdrawn.

Finally, the kinetonucleus becomes shortened and denser, the last remains of the karyosome strand are absorbed, and the animal elongates and regains its normal aspect.

Conclusions.

I have thought that it might be of some interest to compare shortly the above account of division of Trypanoplasma congeri with that given by previous workers for other species of Trypanoplasma. As far as I am aware, the only accounts of division in a trypanoplasma hitherto published are those by Keysselitz, in his paper, "Generations- und Wirtswechsel im Trypanoplasma borreli" (1906), and by Friedrich, in his paper, "Über Bau und Naturgeschichte der Trypanoplasma helicis" (1909). Keysselitz gives on page 28 of his paper five figures of dividing active forms from the blood of the fish, i.e. figs. 12, 14, 22, 23, 24. From these figures it would appear that the process of division in Trypanoplasma congeri shows some difference from that of Trypanoplasma borreli, though, as his series of division seems far from complete, it is quite possible that these differences may be more apparent than real.

(1) As regards the behaviour of the flagella, Keysselitz seems inclined to believe that one of the products of the division keeps the old flagella, and that the other at a comparatively late stage grows out new flagella.

(2) In T. borreli, according to Keysselitz, the trophonucleus divides, showing an internal division centre derived from the karyosome and eight chromosomes, at a stage at which there is no sign of division in the flagella, blepharoplast, or cell body.

(3) The kinetonucleus is said to divide transversely.

The difficult feature in this account of division seems to me the extraordinary amount of variability in the time factor for all these processes; in fact, Keysselitz himself states on page 31: "Den Verlauf der Teilung habe ich bisher in allen seinen einzelnen Phasen im Leben nicht verfolgen können. Wie ich schon oben angegeben habe, trifft man relativ selten sich vermehrende Individuen an. Vorzugsweise sind es Tiere, bei denen die Teilung des chromatischen Apparates und des Plasmas, sowie die Bildung der lokomotorischen Organellen bereits beendet sind und die nur noch mit ihrer hinteren Enden zusammenhangen, eine Phase, die zeitlich längste im Laufe der Teilung zu sein scheint." It is particularly over this last point, however, that a great deal of caution should be exercised. In well-infected smears it is an exceedingly common occurrence to find two trypanoplasma lying in a position which suggests division, but unless there is some absolutely distinctive feature, e.g. as regards the structure of the nuclei, which can be definitely connected with a corresponding structure in an undoubted dividing form, I feel that it is always most hazardous to interpret these appearances as division stages. On the other hand, the differences between the division of Trypanoplasma helicis, as described by Friedrich, and that of Trypanoplasma congeri, seem to be of an absolutely fundamental character. In the first place the karyosome, which is so characteristic a feature of the trophonucleus of most trypanoplasmas, is entirely absent in Trypanoplasma helicis, and in correlation with this fact the division of the trophonucleus appears to consist in a simple constriction of the large vacuolar trophonucleus with its scattered chromatin granules (p. 387). The division of the kinetonucleus is said to be longitudinal (p. 385), but the figures of this process seem hardly convincing. The behaviour of the flagella, again, seems to be very complicated, since it is said on p. 390: "Nachem die für die neue Zelle notwendigen Teile entwickelt sind oder der Anlage nach vorhanden sind, rücken die Kerne und Blepharoplasten auseinander." "Dasselbe geschicht mit den Geisselursprungsstellen, die alsdann im die Nähe des Blepharoplasten verlagert werden. Dabei bildet sich die der alten undulierenden Membran zunächst gelegene Geisselanlage sur vorderen Geissel eines neues Tieres aus, während die der ursprünglichen vorderen Geissel benachbarte zur undulierende Membran des neuen Tieres wird."

It would be seen from the above that there is hardly a single point of agreement between the division of Trypanoplasma congeri and Trypanoplasma helicis, and it would seem almost doubtful whether the two forms can be profitably united in the single genus. It would, I feel, be premature to enter here into a discussion on the comparative morphology of Trypanoplasma congeri and the trypanosomes proper until the rather complicated changes leading up to the resting-stage in the former have been more fully worked out. This I hope to do in a succeeding paper.

Results.

In the division of the active elongate Trypanoplasma congeri the following features are to be noted:

(1) The basal granule divides. This is followed immediately by a splitting of the anterior flagellum, and later, by the splitting of the posterior flagellum and membrane.

(2) The trophonucleus in the first stage enlarges, the intra-nuclear chromatin condensing on the karyosome. The trophonucleus assumes first a spindle and later a dumb-bell shape, which persists to quite a late stage in division. The karyosome appears to act as an internal division centre, and no trace of individual chromosomes can be seen at any stage of division.

(3) The kinetonucleus increases in size and divides by a simple transverse constriction. From its behaviour during division it is, I think, abundantly clear that, at any rate as far as Trypanoplasma congeri is concerned, the kinetonucleus cannot be regarded as a centrosome.

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EXPLANATION OF PLATE 21,

Illustrating Mr. C. H. Martin's paper on "Observations on Trypanoplasma congeri," Part I.

[All the figures were drawn with the camera lucida at table level under a Zeiss 1·5 mm. apochromat. and 18 compensating ocular. For the nomenclature of the structures compare text-figure.]

Fig. 1.—Normal active Trypanoplasma congeri showing flagella, single basal granule, kinetonucleus and trophonucleus with its karyosome and intra-nuclear chromatin granules. A row of faintly marked cytoplasmic granules may be seen passing under the membrane. Flemming, iron-hæmatoxylin, and eosin.

Fig. 2.—Early stage of division. The whole body of the animal is shorter and stouter. The basal granule has divided, the anterior flagellum is split along about a quarter of its length, and the beginning

of the splitting of the posterior flagellum is shown. The kinetonucleus is slightly thicker and the trophonucleus is distinctly enlarged. The intra-nuclear chromatin granules have probably condensed upon the karyosome, which no longer presents the hard outline characteristic of the resting nucleus.

Fig. 3.—The flagella have now split along their whole length. The karyosome has become drawn out into the characteristic dumb-bell-shape within the nuclear membrane. Corrosive acetic, iron-hæmatoxylin, and eosin.

Fig. 4.—The body of the animal has become still shorter. The kinetonucleus is becoming enlarged and losing its intense capacity for nuclear stain. The dividing trophonucleus is almost parallel to the longitudinal axis of the animal's body. Corrosive acetic, iron-hæmatoxylin, and eosin.

Fig. 5.—The body of the animal has become still more deformed. The basal granules with their flagella have shifted apart. The kinetonucleus has become thickened and has now lost its intense capacity for nuclear stains, its lower border is crossed by the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 6.—The basal granules with their flagella now lie at opposite sides of the dividing animal. The lower limb of the enlarged kinetonucleus has adopted its characteristic position at right angles to the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 7.—A slightly later stage than the previous figure, showing the characteristic relations of the enlarged kinetonucleus and the trophonuclear dumb-bell. Flemming, hæmalum, and eosin.

Fig. 8.—A late stage of division. The two products of division are still united by a broad band of cytoplasm, through which the kinetonucleus and trophonuclei still retain their connection. Flemming, hæmalum, and eosin.

Fig. 9.—A recently divided form showing the characteristic rounded shape, the elongate kinetonucleus, and the unabsorbed strand which had connected the trophonuclei. The full length of the flagella are not shown. Corrosive acetic, hæmalum, and eosin.